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Research Article

Co-supplementation of Probiotic- *Bacillus coagulans* SNZ 1969® and Whey Protein Improves Amino Acid Absorption and Reduces the Incidence of Acne in Healthy Adults: A Randomized, Double-blind, Crossover Study

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ABSTRACT

Background: Probiotics enhance absorption of amino acids from protein supplements. This study evaluated the effect of *Bacillus coagulans* SNZ 1969® (probiotic) on amino acid absorption rates and incidence of acneiform lesions after whey protein supplementation.

Methods: Healthy adults aged 18 years-30 years were randomized to receive either whey protein (25 g) and probiotic or whey protein (25 g) alone once daily for 14 days with a 21-day washout period. The primary endpoint was area under the plasma concentration *vs* time curve (AUC0-4h) for amino acids. The secondary endpoints included: C_{max}, T_{max}, incidence of acneiform lesions, and safety.

Results: Of 30 participants, 26 were treated in the first period, of whom, 22 completed the study. The mean (SD) age was 26.0 (3.4) years and the mean (SD) BMI was 24.0 (3.0) kg/m^2. Adding probiotic to whey protein numerically increased the total plasma amino acid concentration (AUC0-4h), however the difference was not statistically significant. The C_{max} of arginine, cysteine, and histidine significantly increased with probiotic ($p<0.05$). Probiotic supplementation led to a numerically faster T_{max} for most amino acids; though not statistically significant. The incidence of acneiform lesions was significantly lower in the probiotic *vs* protein group (13.6% *vs* 54.5%; $p<0.05$). No treatment related adverse events were reported.

Conclusion: Adding *B. coagulans* SNZ 1969® to whey protein significantly improved the C_{max} for certain amino acids and reduced the incidence of acneiform lesions. The probiotic was safe and well tolerated, and beneficial in reducing acneiform lesions for those consuming whey protein supplements.

Keywords: Whey protein, Probiotic, Pharmacokinetics, Acne vulgaris

INTRODUCTION

Probiotics are defined as live microorganisms which, when consumed in adequate amounts confer a health benefit to the host [1,2]. They are associated with to a host of healthful benefits and outcomes including modulation of gut microbiota, bolster gut barrier function, and boost overall immunity [3,4]. Probiotics can also limit pathogen adhesion to host tissue and modulate the production of vitamins, short-chain fatty acids, and neurotransmitters involved in gut-brain communication [5]. Furthermore, probiotics have been shown to impact the absorption of key nutrients and alter the production of various forms of digestive enzymes [5].

Bacillus coagulans SNZ 1969® is a rod-shaped, slightly acidophilic, gram-positive, spore-forming, highly resilient, lactic acid-producing bacteria [6,7]. It is the most widely studied strain of *B. coagulans* and has been identified as safe for human consumption by the United States Food and Drug Administration (USFDA) and the European Food Safety Authority (EFSA) and is included in the list of Generally Recognized as Safe (USFDA number GRN-597) and Qualified Presumption of Safety (QPS) [6,8]. The efficacy and safety of *B. coagulans* SNZ 1969® have been demonstrated in various studies, highlighting its potential to improve gut health and nutrient absorption [6-8].

Whey protein, derived from milk, is the most common protein supplement used to gain muscle mass and strength by young

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people and athletes [9-11]. It is a rich source for Essential Amino Acids (EAAs), sulfur-containing amino acids, and Branched Chain Amino Acids (BCAAs) which provide substrates for protein synthesis and thereby support muscle growth [9,12]. Whey proteins extract or concentrate contains various growth factors including Insulin-like Growth Factor (IGF)-I and II, Transforming Growth Factor (TGF), Platelet Derived Growth Factor (PDGF), and Fibroblast Growth Factor (FGF)-1 and -2 which are responsible for the insulin tropic effects of milk may contribute more to acne development than the actual fat or dairy content [13,14].

An untoward effect of protein supplementation is the increased incidence of Acne Vulgaris. In a prospective observational study, Pontes et al. assessed the relationship between the use of protein-calorie supplements, such as whey protein, and onset or exacerbation of acne vulgaris in young adults, and found an association between acne onset or exacerbation with progressive use of protein-calorie supplements in females and in those with no history of acne [14]. A recent case-controlled study that investigated the association between consumption of whey protein supplement and development of acne among Jordanian athletes and young adults has shown a 2.9-fold higher odds of having acne in participants who consume whey protein compared to controls [15].

Acne vulgaris is a common inflammatory skin disease of pilosebaceous units that is characterized by the formation of comedones, papules, pustules, cysts, nodules, and scars that appear mainly on the face, upper trunk, and sometimes extremities [3,4,13,16]. Globally, it is the 8th most prevalent disease accounting for 9.4% of the population (8.96% in men and 9.81% in women), of which 85% are young adults [17-19]. Acne vulgaris has shown to significantly impair the quality of life, affecting the mental health often leading to social isolation, depression, anxiety, and in extreme cases suicidal ideation, thereby increasing the disease burden [3,20,21].

Earlier studies which assessed the effect of addition of probiotics on the rate and extent of amino acids absorption following animal or plant protein ingestion have shown mixed results with some studies showing significant increase in the area under the plasma concentration *vs* time curve (AUC) and peak plasma concentration (C_{max}) of amino acids with probiotic administration while other studies have shown no difference in the plasma amino acid levels of leucine, BCAA, EAA, and total amino acids between the groups [5,22,23]. While the effects of probiotics are often shared by those of the same genus, many effects are strain-specific, and it is important to determine which probiotic strains may improve protein kinetics. Additionally, though whey protein is generally considered the standard with regards to protein quality and is the most widely consumed protein, limited studies examined the effects of probiotics on whey protein digestion. Therefore, this study aims to evaluate the rate and extent of amino acid absorption and incidence of acneiform lesions after the ingestion of whey protein along with probiotic *B. coagulans* SNZ 1969® in healthy human subjects.

METHODS

Study design and participants

This was a randomized, double-blind, crossover trial involving healthy human subjects. Adult (male and female) participants aged 18 years-30 years, weighing at least 50 kg, and Body Mass Index (BMI) between 18.5 kg/m^2 and 30.0 kg/m^2; with acceptable findings during study registration and screening including medical history, physical examination, laboratory evaluations, 12-lead Electrocardiogram (ECG) and chest X-Ray; non-smokers and non-alcoholics; and those completing at least 30 minutes of moderate exercise three days per week were included. Subjects with history of hypersensitivity to whey protein or related group of drugs; history or presence of asthma, urticaria, or other allergic-type reactions after taking aspirin or other NSAIDs; history of seizures, diabetes, migraine, hypertension, cardiovascular, pulmonary, neurological or psychiatric disorder, dermatological, endocrine, eye disorders, immunological, hepatic, renal, hematopoietic, metabolic, gastrointestinal, ongoing infectious diseases, or any other significant abnormality as evidenced by medical history and physical examination or according to the opinion of the physician were excluded.

Participants were randomized (1:1) to receive either Treatment A (25 g whey protein concentrate containing not less than [NLT] 2 billion Colony Forming Units [CFU] of *B. coagulans* SNZ 1969® or Treatment B (25 g whey protein alone [amino acid composition presented in Table S1]). Each participant received a single daily dose of investigational product (treatment A or treatment B) for a period of 2 weeks as per the randomization schedule, separated by a washout period of 21 days and then crossover to the other treatment arm (Figure 1).

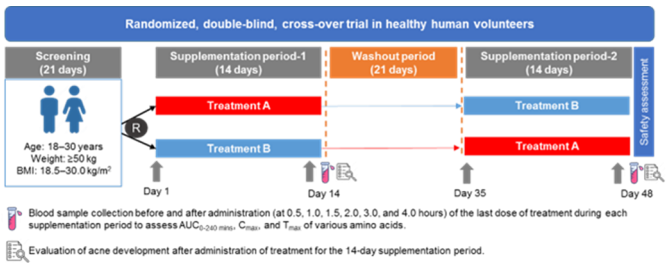

Figure 1: Study Design Treatment A: Whey protein+probiotic *Bacillus coagulans* SNZ 1969®; Treatment B: Whey protein. AUC, area under the plasma concentration *vs* time curve; BMI, body mass index; C_{max}, maximum (peak) plasma concentration; R, randomization; T_{max}, time taken to achieve peak plasma concentration

Informed consent was obtained from all the subjects prior to enrollment into the study. The study protocol, informed consent form, and other study related documents were approved by

independent ethics committee (MAARG, Hyderabad). The study was conducted in accordance with the IEC approved protocol and other study related documents and SOPs, and other pertinent requirements of Declaration of Helsinki (Brazil, October 2013), ICH [E6(R2)] 'Good Clinical Practice' guidelines, "ICMR Ethical Guidelines for Biomedical Research on Human Subjects" 2017, "CDSCO Guidelines for BA/BE Studies", and "New Drugs and Clinical Trial Rules, (Third Amendment) Rules 2022. This study was registered with the Clinical Trials Registry of India-CTRI/2024/01/061065 (Registered on: 02/01/2024) and was registered prospectively before initiating screening of the subjects (prior to enrolment).

Study treatments and supplementation procedure: Participants were randomized to receive whey protein with or without probiotic *B. coagulans* SNZ 1969® (Treatment A or Treatment B). Participants assigned to treatment A received 25 g of whey protein concentrate containing NLT 2 billion CFU of *B. coagulans* SNZ 1969® (Manufactured by Sanzyme Biologics Private Limited). The treatment was self-administered by subjects orally once daily with 300 mL of water at ambient temperature under fasting conditions (and ingest between 08:00 am to 10:00 am at least 1 hour prior to breakfast) in each study period. Likewise, participants assigned to treatment B received 25 g of whey protein concentrate (Manufactured by Mullins Whey Inc., Mosinee, WI 54455) once daily with 300 mL of water under fasting conditions. The study participants were instructed to ingest the provided dose of treatments at same time during the study periods, and required to complete a supplementation log to document when each dose of their assigned treatment was consumed.

Participants visited the clinical facility on Day 0 (for randomization and supply of treatments for the first 13 days of the treatment period) and Day 14 (for administration of the 14th dose, blood sample collection, and evaluation of acne development) during the supplementation period 1. Similarly, during the supplementation period 2 (following completion of 21-day washout period), participants visited the clinical facility for supply of treatments on Day 34, and on Day 48 for administration of the 14th dose, blood sample collection, and assessment of acne development. Daily diet was recorded and subjects were asked to repeat the same diet during the supplementation period along with completion of 30 minutes of moderate exercise three days per week.

Blood sampling and processing: In each supplementation period, a total of seven blood samples were collected from all available participants at predefined time intervals-prior to ingestion of the final dose of the assigned treatment and at 0.5 hour, 1.0 hour, 1.5 hours, 2.0 hours, 3.0 hours, and 4.0 hours after ingestion. After collection, the blood samples were placed in an ice bath until centrifugation. Blood samples were centrifuged within 45 minutes of sample collection and spun at 3500 rpm at 4°C for 10 minutes. The plasma was separated and stored in a deep freezer at -20°C ± 15°C until analyzed. Estimation of individual amino acids was done using LC-MS based method.

Outcomes and assessments: The primary outcome of the study was to assess the area under the plasma concentration versus time curve (AUC [0 h-4 h]) for the different amino acids. Secondary outcomes were the maximum (peak) concentrations achieved in plasma (C_{max}), time taken to achieve peak plasma concentrations (T_{max}) for the different amino acids, incidence of acne, and safety. The area under the plasma concentration-time curve was calculated by linear trapezoidal method and the individual and mean plasma concentration *vs* time plots were generated on both linear and semi-log plots for different amino acids. The pharmacokinetic parameters (AUC [0 h-4 h], C_{max}, and T_{max}) were estimated using Phoenix® WinNonlin® version 8.4.

Dermatological evaluation was performed to investigate if subjects experience any acne development or that worsens during the study in different study periods. Acne grading was performed at baseline (Day 0), end of protein supplementation period 1 (Day 14), prior to start of protein supplementation period 2 (Day 34), and at the end of protein supplementation period 2 (Day 48). High resolution photographs were taken at each of the above mentioned time points for acne grade assessment. A qualified dermatologist performed the assessment using the Acne Severity Grading Scale (Table S2).

Subjects were monitored for Adverse Events (AEs) throughout the study duration. Vital signs (seated blood pressure, respiratory rate, radial pulse rate, and body temperature) and well-being of the subject was assessed on Day 0, Day 14, Day 34, and Day 48. At the end of the study, post study safety evaluation was performed which included hematology and biochemical tests. The association between the incidence of AEs and study medication was established using WHO-UMC system for standardized case causality assessment.

Statistical analyses

All the results are presented descriptively. Continuous variables were presented as mean and standard deviation. Before any statistical tests, the normality was assessed for all dependent variables. All non-normal data was log transformed and then analyzed using both parametric and non-parametric approaches. All reported P values were computed using parametric approaches. Paired sample t-tests were used to determine between-group differences for the AUC (0 h-4 h), C_{max}, and T_{max} values for all individually measured amino acids. Statistical analysis was performed using SAS®, Version 9.4 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Participant disposition and demographic characteristics

Of 30 subjects randomized, 26 were enrolled into the supplementation period 1. All enrolled subjects (n=26) were dosed in period 1 and 22 subjects were dosed in period 2. 22

subjects completed both the treatment periods of the study and were considered for pharmacokinetic, biochemical, and statistical analysis (Figure 2). The mean (SD) age of enrolled participants was 26.0 (3.4) years, mean (SD) body weight was 64.7 (10.8) kg, and the mean (SD) BMI was 24.0 (3.0) kg/m^2.

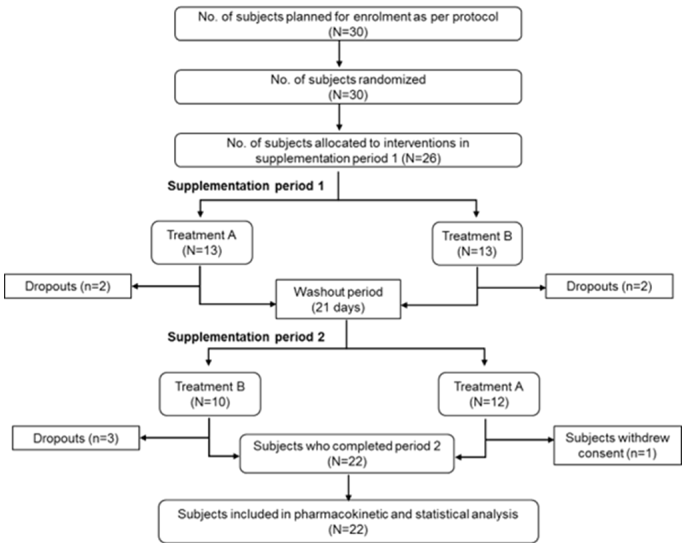

Figure 2: CONSORT flow diagram (Patient disposition flowchart) Treatment A consists of whey protein and probiotic (*Bacillus coagulans* SNZ 1969®), while treatment B consists of whey protein alone

Area under the plasma concentration vs time curve (AUC µmol/L 4 hours): Addition of *B. coagulans* SNZ 1969® to whey protein numerically increased the mean plasma concentration of all amino acids except aspartic acid (Figure 3). The area under the plasma concentration *vs* time curve (AUC 0 h-4 h) of all amino acids except aspartic acid was numerically higher in the whey protein+*B. coagulans* SNZ 1969® group compared with the whey protein group, but the between group difference was not statistically significant (Table 1). The AUC of aspartic acid was slightly higher in the whey protein group versus whey protein and *B. coagulans* SNZ 1969® group (mean AUC: 6.50 µmol/L *vs* 5.65 µmol/L; Table 1). The mean AUC of Branched Chain Amino Acids (BCAA), essential amino acids (EAA), and total amino acids was numerically higher in the whey protein+*B. coagulans* SNZ 1969® group compared with the whey protein group (mean AUC of BCAA: 3415.77 µmol/L *vs* 3407.67 µmol/L; EAA: 6336.58 *vs* 6140.50 µmol/L; and total amino acids: 12557.26 *vs* 12117.65 µmol/L).

Table 1: Individual amino acids, branched chain amino acids, essential amino acids, and total amino acids area under the curve (AUC, µmol/L 4 hours)

Amino acid (s)	Treatment A (Whey protein+BC SNZ 1969®)		Treatment B (Whey protein alone)		Difference (%)	95% CI	p-value
	Mean (µmol/L)	SD	Mean (µmol/L)	SD			
Alanine#	2264.27	505.22	2205.69	452.80	2.66	-262.65, 379.82	0.7083
Arginine#	511.71	139.73	477.47	114.75	7.17	-18.39, 86.87	0.1904
Aspartic acid@	5.65	8.67	6.50	8.07	-13.08	-10.68, 8.98	0.8491
Cysteine#	86.83	19.95	77.07	21.34	12.66	-0.05, 19.57	0.0510
Glutamic acid#	202.75	106.21	194.47	114.01	4.26	-28.29, 44.86	0.6426
Glycine#	1112.62	469.55	1069.38	520.16	4.04	-287.34, 373.80	0.7883
Histidine#	255.23	108.66	247.12	93.55	3.28	-37.24, 53.47	0.7135
Isoleucine#	724.63	210.01	723.59	181.30	0.14	-142.30, 144.36	0.9882
Leucine#	1215.65	306.81	1211.21	183.08	0.37	-157.48, 166.35	0.9551
Lysine#	948.57	261.00	876.37	261.20	8.24	-74.90, 219.29	0.3190
Methionine#	185.10	45.46	173.40	36.86	6.74	-7.89, 31.29	0.2281
Phenyl alanine#	264.92	52.42	262.30	46.30	1.00	-22.55, 27.80	0.8305
Proline#	1022.87	207.90	968.10	205.53	5.66	-40.44, 149.97	0.2449
Serine#	654.00	175.67	640.65	150.30	2.08	-68.19, 94.89	0.7369
Threonine#	992.55	307.56	927.52	374.24	7.01	-69.84, 199.90	0.3274
Tryptophan#	274.44	69.78	246.12	51.03	11.51	-5.93, 62.56	0.1002
Tyrosine#	356.86	95.65	340.10	62.35	4.93	-24.11, 57.62	0.4034
Valine#	1475.50	334.33	1472.86	280.46	0.18	-214.53, 219.80	0.9801
Branched chain amino acids#	3415.77	798.84	3407.67	615.89	0.24	-484.90, 501.11	0.9731

Essential amino acids#	6336.58	1322.75	6140.50	1157.00	3.19	-553.07, 945.24	0.5920
Total amino acids#	12557.26	2481.36	12117.65	2423.80	3.63	-1080.49, 1959.71	0.5540

#indicates non-significant increase in AUC (p>0.05) in the BC SNZ 1969®+whey protein group versus whey protein group. @ indicates non-significant decrease in AUC (p>0.05) in the BC SNZ 1969®+whey protein group versus whey protein group. Branched chain amino acids include Valine, Isoleucine, and Leucine. Essential amino acids include Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine. Total amino acids in whey protein include Alanine, Arginine, Aspartic acid, Cysteine, Glutamic acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, and Valine.

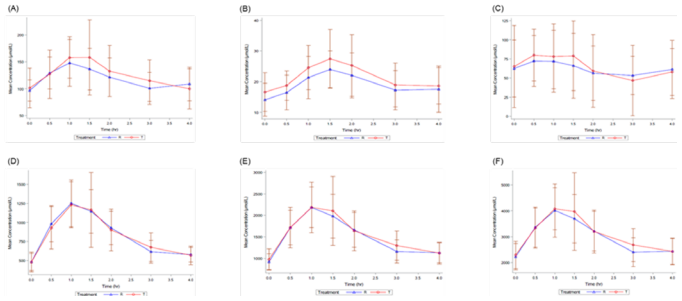

Figure 3: Mean amino acid concentration versus time curve for arginine (a), cysteine (b), histidine (c), branched chain amino acids (d), essential amino acids (e), and total amino acids (f). Error bars indicate standard deviation. R in the figures refers to reference group (i.e., Whey protein group) and T refers to the Treatment group (i.e., Whey protein+probiotic *Bacillus coagulans* SNZ 1969® group). Branched chain amino acids include Valine, Isoleucine, and Leucine. Essential amino acids include Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine. Total amino acids in whey protein include Alanine, Arginine, Aspartic acid, Cysteine, Glutamic acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, and Valine

Maximum amino acids concentration in plasma (C_{max}, µmol/L): Addition of *B. coagulans* SNZ 1969® to whey protein led to statistically significant increase in the mean peak plasma concentration (C_{max}) of 3 amino acids namely arginine (mean C_{max} 183.23 *vs* 156.95 µmol/L; Δ [95% CI]: 16.74% [1.58-50.96]; p=0.0381), cysteine (32.01 µmol/L *vs* 26.10 µmol/L; Δ [95% CI]: 22.62% [0.30-11.51]; p=0.0400), and histidine (125.75 *vs* 104.34 µmol/L; Δ [95% CI]: 20.52% [0.03-42.80]; p=0.0497). The C_{max} of BCAA, EAA, and total amino acids was numerically higher in the whey protein+probiotic group versus whey protein group (BCAA: 1347.68 *vs* 1312.82 µmol/L; EAA: 2484.62 µmol/L *vs* 2358.77 µmol/L; total amino acids: 4615.46 µmol/L *vs* 4328.93 µmol/L). Except glutamic acid, the C_{max} of all amino acids was numerically higher in the whey protein+*B. coagulans* SNZ 1969® group compared with the whey protein group (Table 2).

Table 2: Maximum concentration (C_{max}, µmol/L) of individual amino acids, branched chain amino acids, essential amino acids, and total amino acids

Amino acid (s)	Treatment A (Whey protein+BC SNZ 1969®)		Treatment B (Whey protein alone)		Difference (%)	95% CI	p-value
	Mean (µmol/L)	SD	Mean (µmol/L)	SD			
Alanine#	764.18	234.41	715.64	167.71	6.78	-82.98, 180.07	0.4513
Arginine*	183.23	63.10	156.95	39.27	16.74	1.58, 50.96	0.0381
Aspartic acid#	5.37	5.33	4.70	4.18	14.35	-4.17, 5.52	0.7604
Cysteine*	32.01	10.69	26.10	6.81	22.62	0.30, 11.51	0.0400
Glutamic acid@	80.20	40.62	80.28	41.97	-0.11	-13.83, 13.66	0.9899
Glycine#	359.00	174.18	344.55	137.47	4.20	-80.09, 109.00	0.7537
Histidine*	125.75	41.27	104.34	32.87	20.52	0.03, 42.80	0.0497
Isoleucine#	314.59	127.91	305.27	90.54	3.05	-68.83, 87.47	0.8066
Leucine#	516.23	173.44	506.55	102.82	1.91	-83.81, 103.18	0.8316
Lysine#	380.27	131.23	344.59	113.07	10.35	-39.86, 111.22	0.3371
Methionine#	73.18	24.30	65.00	16.99	12.58	-3.29, 19.65	0.1531
Phenyl alanine#	94.36	25.82	89.26	18.16	5.71	-6.72, 16.92	0.3802

Proline#	347.73	106.32	314.59	64.70	10.53	-16.30, 82.57	0.1779
Serine#	233.36	88.86	215.27	53.31	8.40	-23.76, 59.95	0.3789
Threonine#	365.36	121.79	358.23	165.83	1.99	-56.97, 71.25	0.8192
Tryptophan#	98.01	29.19	84.53	21.11	15.95	-1.58, 28.54	0.0767
Tyrosine#	124.73	39.63	113.60	27.52	9.80	-8.33, 30.60	0.2476
Valine#	516.86	182.66	501.00	108.49	3.17	-88.08, 119.80	0.7541
Branched chain amino acids#	1347.68	470.23	1312.82	283.42	2.66	-230.28, 300.01	0.7872
Essential amino acids#	2484.62	717.26	2358.77	520.51	5.34	-272.42, 524.12	0.5182
Total amino acids#	4615.46	1347.76	4328.93	910.92	6.62	-435.43, 1008.49	0.4184

*indicates significant increase in C_{max} ($p<0.05$) in the BC SNZ 1969®+whey protein group versus whey protein group; #indicates non-significant increase in C_{max} ($p>0.05$) in the BC SNZ 1969®+whey protein group versus whey protein group; @indicates non-significant decrease in C_{max} ($p>0.05$) in the BC SNZ 1969®+whey protein group versus whey protein group. Branched chain amino acids include Valine, Isoleucine, and Leucine. Essential amino acids include Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine. Total amino acids in whey protein include Alanine, Arginine, Aspartic acid, Cysteine, Glutamic acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, and Valine.

Time to achieve maximum plasma concentration (T_{max}): Addition of *B. coagulans* SNZ 1969® to whey protein led to a faster achievement of maximum concentrations with expedited T_{max} for all measured amino acids except cysteine, glycine, leucine, phenyl alanine, and Serine. The T_{max} of all amino acids (except cysteine, glycine, leucine, phenyl alanine, and serine) was numerically quicker (i.e., shorter time to achieve C_{max}) in the whey protein+*B. coagulans* SNZ 1969® group than whey protein group; however, the difference between groups was not statistically significant (Table 3). Similar findings were observed between groups for the BCAA (mean T_{max}: 1.06 *vs* 1.09 h), EAA (1.13 h *vs* 1.23 h), and total amino acids (1.13 h *vs* 1.21 h).

Table 3: Time to maximum concentration (T_{max}) of individual amino acids, branched chain amino acids, essential amino acids, and total amino acids

Amino acid(s)	Treatment A (Whey protein+BC SNZ 1969®)		Treatment B (Whey protein alone)		Difference (%)	95% CI	p-value
	Mean (hours)	SD	Mean (hours)	SD			
Alanine#	1.11	0.49	1.14	0.76	-2.00	-0.43, 0.38	0.9088
Arginine#	1.11	0.46	1.39	0.93	-19.88	-0.75, 0.20	0.2364
Aspartic acid#	1.10	0.39	1.15	0.67	-4.35	-0.54, 0.44	0.8227
Cysteine@	1.68	0.82	1.68	0.88	0.05	-0.49, 0.49	0.9969
Glutamic acid#	1.00	0.49	1.28	0.91	-21.58	-0.72, 0.16	0.2072
Glycine@	1.11	0.98	1.00	0.95	11.36	-0.48, 0.71	0.6958
Histidine#	1.36	0.90	1.84	1.35	-25.93	-1.23, 0.28	0.2023
Isoleucine#	1.02	0.29	1.09	0.25	-6.25	-0.23, 0.09	0.378
Leucine@	1.09	0.29	1.09	0.29	0.00	-0.15, 0.15	1.0000
Lysine#	1.09	0.29	1.20	0.48	-9.43	-0.35, 0.12	0.3287
Methionine#	1.02	0.36	1.09	0.29	-6.25	-0.25, 0.12	0.4514
Phenyl alanine@	1.05	0.43	0.86	0.32	21.05	-0.05, 0.41	0.1187
Proline#	1.21	0.43	1.25	0.37	-3.56	-0.26, 0.17	0.6722
Serine@	1.05	0.34	1.02	0.42	2.22	-0.19, 0.23	0.8246
Threonine#	1.23	0.65	1.36	0.60	-10.00	-0.51, 0.23	0.4514
Tryptophan#	1.25	0.37	1.41	0.50	-11.23	-0.44, 0.12	0.2489
Tyrosine#	1.05	0.38	1.09	0.37	-4.08	-0.26, 0.17	0.6722

Valine#	1.07	0.39	1.09	0.29	-2.41	-0.21, 0.16	0.7700
Branched chain amino acids#	1.06	0.29	1.09	0.25	-2.89	-0.17, 0.11	0.6435
Essential amino acids#	1.13	0.28	1.23	0.26	-7.84	-0.23, 0.04	0.1630
Total amino acids#	1.13	0.31	1.21	0.23	-6.49	-0.24, 0.09	0.3338

#indicates non-significant decrease in the T_{max} ($p>0.05$) in the BC SNZ 1969®+whey protein group versus whey protein group. @indicates non-significant increase or no change in the T_{max} ($p>0.05$) in the BC SNZ 1969®+whey protein group versus whey protein group. Branched chain amino acids include Valine, Isoleucine, and Leucine. Essential amino acids include Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine. Total amino acids in whey protein include Alanine, Arginine, Aspartic acid, Cysteine, Glutamic acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, and Valine.

Incidence of acneiform lesions: Of 26 subjects who completed supplementation period I, 19 were males and 7 were females. In the whey protein group, 7 of 13 subjects who received whey protein alone in supplementation period I developed acne (mild/moderate) with an incidence rate of 53.8%, while in the *B. coagulans* SNZ 1969®+whey protein group, 2 of 13 subjects developed acne (mild/moderate) with an incidence rate of 15.4% showing a significant reduction in the incidence rate with *B. coagulans* SNZ 1969® supplementation ($p<0.05$; Table 4). Of 22 subjects who completed supplementation period II, 18 were males and 4 were females. In the whey protein group, 5 of 10 subjects developed acne (mild/moderate) with an incidence rate of 50.0%, while in the *B. coagulans* SNZ 1969®+Whey protein group, only 1 of 12 subjects developed acne (mild/moderate) with an incidence rate of 8.3% demonstrating a significant reduction ($p<0.05$) in the incidence rate with *B. coagulans* SNZ 1969® supplementation (Table 4).

Table 4: Incidence of acneiform lesions in the whey protein+*Bacillus coagulans* SNZ 1969® group versus whey protein group

Grade	Severity	Supplementation Period I			
		Bacillus coagulans SNZ 1969®+Whey protein (n=13)		Whey protein (n=13)	Incidence
		Male (n=10)	Female (n=3)	Male (n=9)	Female (n=4)
I	Mild	1	-	4	1
II	Moderate	-	1	1	1
III	Moderately severe	-	-	-	-
IV	Severe	-	-	-	-
Grade	**Severity**	**Supplementation Period II**			
		Bacillus coagulans SNZ 1969®+Whey protein (n=12)		Whey protein (n=10)	Incidence
		Male (n=8)	Female (n=2)	Male (n=10)	Female (n=2)
I	Mild	1	-	3	-
II	Moderate	-	-	1	1
III	Moderately severe	-	-	-	-
IV	Severe	-	-	-	-

In total, of the 22 participants who completed the study, 12 participants (54.5%; 9 male and 3 female) in the whey protein group developed acne, while only 3 participants (13.6%; 2 male and 1 female) in the whey protein and *B. coagulans* SNZ 1969® group developed acne. Addition of probiotic *B. coagulans* SNZ 1969® to whey protein significantly reduced the incidence of acne ($p<0.05$). This effect was more prominent in females and in individuals without current acne and no family history of acne.

Safety: Overall, no treatment related AEs were reported during the study and post study safety assessment. No serious AEs or deaths, and no significant change in vital signs were reported during the study.

DISCUSSION

This randomized trial evaluated the amino acid concentrations (i.e., the rate and extent of amino acid absorption into blood stream) and acne incidence following the ingestion of whey protein with and without probiotic and found that *B. coagulans* SNZ 1969® supplementation increased the total plasma concentrations of most amino acids, significantly increased the C_{max} of some amino acids-arginine, cysteine, and histidine, led to rapid attainment of C_{max} (faster T_{max}) of most amino acids,

and significantly reduced the incidence of acneiform lesions in adult healthy participants. The findings observed in this study are similar to what was observed in a randomized trial that examined the effect of 28 days of probiotic (Bacillus subtilis DE111) supplementation on plasma amino acid appearance after whey protein ingestion and showed no difference in the plasma amino acid levels of leucine, BCAA, EAA, and total amino acids between the groups [23]. In contrast to the findings, a few other studies have reported a significant increase in amino acid concentrations and AUC following administration of probiotics [5,22]. In a randomized, double-blind, crossover study, Stecker et al. examined the impact of probiotic *B. coagulans* GBI-30, 6086 (BC30) supplementation on milk protein digestion and blood amino acid concentrations and found that adding BC30 to milk protein significantly improved the AUC of amino acids arginine and isoleucine, increased the C_{max} of arginine, serine, ornithine, methionine, glutamic acid, phenylalanine, isoleucine, tyrosine, EAA, and total amino acids, and aided in faster attainment of C_{max} (i.e., shorter T_{max}) for amino acids glutamine, citruline, threonine, and alanine [5]. In another study, Jager et al investigated the effect of Amino Alta™ supplementation, a multi-strain probiotic containing 5 billion CFU of *Lactobacillus paracasei* LP-DG® (CNCM I-1572) plus 5 billion CFU *L. paracasei* LPCS01(DSM 26760), on plant protein (pea protein) absorption on blood amino acid concentrations and found that probiotic administration significantly increased the C_{max} and AUC of methionine, histidine, valine, leucine, isoleucine, tyrosine, BCAA, and EAA without significantly changing the T_{max} [22].

In this study, supplementation of probiotic *B. coagulans* SNZ 1969® significantly increased the C_{max} of arginine, cysteine, and histidine. Arginine is a semi essential amino acid that plays a crucial role in many cellular functions including modulation of immune function, wound healing, hormone secretion, vascular tone, insulin sensitivity, and endothelial function [24]. Histidine is an essential amino acid with unique amphoteric properties making it a key catalytic residue in many enzymes [25,26]. Histidine serves as a precursor for several hormones, carnosine in human muscle and parts of the brain where carnosine acts as a buffer and antioxidant, and also a precursor for the neurotransmitter histamine, which is important for mediating growth and functionality of immune cells [25-28]. Histidine also performs anti-inflammatory, anti-oxidant, and anti-secretory functions [26,28]. Cysteine is a sulfur containing amino acid with diverse roles in protein function and oxidative metabolism [28]. The thiol group of cysteine is responsible for a number of important functions including formation of disulfide bonds that define the structures of many proteins, stabilizing extracellular proteins, conferring proteolytic resistance, and enabling catalytic properties of enzymes [28]. Cysteine also serves as a precursor or contributes to the synthesis of intracellular metabolites-taurine, coenzyme A, and glutathione [28].

Most of the protein supplements including whey protein are rich in growth factors and have a propensity to cause acneiform lesions and are proven to be comedogenic [9,14,15]. A systematic literature review that investigated the potential AEs of whey protein supplementation has shown that indiscriminate or chronic use may cause some AEs specifically on kidney and liver function [11]. In addition, the study showed that supplementation of whey protein increases the presence of acne, aggravation of aggression, and modification of microbiota [11]. In another case-controlled study, Muhaidat et al. examined the association of whey protein supplements on acne risk among adolescents and adults and showed a direct association between whey protein consumption and acne risk [15].

Previous clinical studies that examined the effect of oral probiotics on acne incidence or exacerbations have demonstrated that ingestion of probiotics reduced the number of acneiforms papules and pustules through a variety of mechanisms including anti-inflammatory and bactericidal properties reduction of IGF-1 and increase in the forkhead box protein O1 (FOXO1) gene expression, increase in levels of anti-inflammatory cytokine (interleukin-10), modifying the barrier function of the skin, inducing the production of healthy ceramides, or immunomodulatory effects on keratinocytes and epithelial cells [29-34]. A recent randomized, placebo-controlled trial that investigated the efficacy and safety of an adjuvant probiotic preparation (*Lacticaseibacillus rhamnosus* (CECT 30031) and cyanobacterium Arthrospira platensis (BEA_IDA_0074B) on the clinical course of acne has shown that ingestion of probiotics significantly reduced the number of non-inflammatory acne lesions (-18.6% *vs* -10.5%, p=0.03) and increased the proportion of patients with improvement in Global Acne Grading System (42.5% *vs* 20.6%, p=0.02). The incidence of AEs was similar between the groups and the probiotic administered was effective and well tolerated by the patients [35].

In this study, the addition of probiotic *B. coagulans* SNZ 1969® to whey protein has led to a significant reduction (13.6% *vs* 54.5%) in the proportion of patients developing acne. The effect was more prominent in females and in individuals without current acne and no family history of acne. The possible mechanism by which the probiotic exerted this effect is through its antimicrobial activity reducing sebum secretion with a potential role in controlling seborrheic conditions like acne vulgaris. The treatment was well tolerated by subjects and were found to be safe. The key strength of the study centers on the randomized, double-blind, crossover design with an isocaloric control group and a study population that was representative of healthy men and women. Moreover, the two-week supplementation period used in this study has been shown to be sufficient for the ingested probiotic to exert physiological outcomes, similar to other studies of this nature.

CONCLUSION

In summary, results from this study demonstrate that addition

of probiotic *B. coagulans* SNZ 1969® to whey protein has shown better and faster amino acid absorption, as evidenced from the pharmacokinetic parameters (AUC0-4 h, C_{max} and T_{max}) and significantly reduced the incidence of acneiform lesions in adult healthy subjects. These findings suggest that addition of probiotic *B. coagulans* SNZ 1969® to the whey protein makes it less comedogenic as compared to whey protein supplementation alone.

CONFLICT OF INTEREST

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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